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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA,

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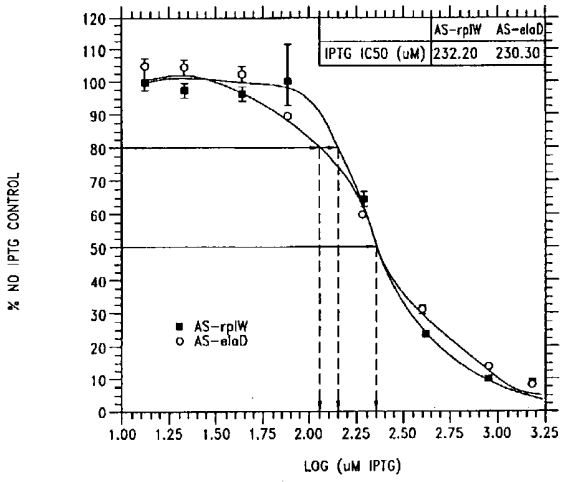
(54) Title: IDENTIFICATION OF ESSENTIAL GENES IN MICROORGANISMS



(57) Abstract: The sequences of antisense nucleic acids which inhibit the proliferation of prokaryotes are disclosed. Cell-based assays which employ the antisense nucleic acids to identify and develop antibiotics are also disclosed. The antisense nucleic acids can also be used to identify proteins required for proliferation, express these proteins or portions thereof, obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate molecules for rational drug discovery programs. The nucleic acids can also be used to screen for homologous nucleic acids that are required for proliferation in cells other than Staphylococcus aureus, Salmonella typhimurium, Klebsiella pneumoniae, and Pseudomonas aeruginosa. The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation required genes in other organisms.

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(54) **IDENTIFICATION OF ESSENTIAL GENES IN MICROORGANISMS**

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(22) Filed: **Oct. 25, 2002**

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(52) **U.S. Cl.** **435/6**; 435/69.1; 435/183; 435/252.33; 435/320.1; 530/350; 536/23.2; 435/325; 435/254.2; 435/419

(57) **ABSTRACT**

The sequences of antisense nucleic acids which inhibit the proliferation of prokaryotes are disclosed. Cell-based assays which employ the antisense nucleic acids to identify and develop antibiotics are also disclosed. The antisense nucleic acids can also be used to identify proteins required for proliferation, express these proteins or portions thereof, obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate molecules for rational drug discovery programs. The nucleic acids can also be used to screen for homologous nucleic acids that are required for proliferation in cells other than *Staphylococcus aureus, Salmonella typhimurium, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation required genes in other organisms.

ID ACA29828 standard; DNA; 483 BP.
XX
AC ACA29828;
XX
DT 19-JUN-2003 (first entry)
XX
DE Prokaryotic essential gene #11485.
XX
KW Antisense; ds; prokaryotic essential gene; cell proliferation;
KW drug design; gene.
XX
OS Corynebacterium diphtheriae.
XX
PN WO200277183-A2.
XX
PD 03-OCT-2002.
XX
PF 21-MAR-2002; 2002WO-US009107.
XX
PR 21-MAR-2001; 2001US-00815242.
PR 06-SEP-2001; 2001US-00948993.
PR 25-OCT-2001; 2001US-0342923P.
PR 08-FEB-2002; 2002US-00072851.
PR 06-MAR-2002; 2002US-0362699P.
XX
PA (ELIT-) ELITRA PHARM INC.
XX
PI Wang L, Zamudio C, Malone C, Haselbeck R, Ohlsen KL, Zyskind JW;
PI Wall D, Trawick JD, Carr GJ, Yamamoto R, Forsyth RA, Xu HH;
XX
DR WPI; 2003-029926/02.
DR P-PSDB; ABU25958.
XX
PT New antisense nucleic acids, useful for identifying proteins or screening
PT for homologous nucleic acids required for cellular proliferation to
PT isolate candidate molecules for rational drug discovery programs.
XX
PS Claim 14; SEQ ID NO 17698; 1766pp; English.
XX
CC The invention relates to an isolated nucleic acid comprising any one of
CC the 6213 antisense sequences given in the specification where expression
CC of the nucleic acid inhibits proliferation of a cell. Also included are:
CC (1) a vector comprising a promoter operably linked to the nucleic acid
CC encoding a polypeptide whose expression is inhibited by the antisense
CC nucleic acid; (2) a host cell containing the vector; (3) an isolated
CC polypeptide or its fragment whose expression is inhibited by the
CC antisense nucleic acid; (4) an antibody capable of specifically binding
CC the polypeptide;(5) producing the polypeptide; (6) inhibiting cellular
CC proliferation or the activity of a gene in an operon required for
CC proliferation; (7) identifying a compound that influences the activity of
CC the gene product or that has an activity against a biological pathway
CC required for proliferation, or that inhibits cellular proliferation; (8)
CC identifying a gene required for cellular proliferation or the biological
CC pathway in which a proliferation-required gene or its gene product lies
CC or a gene on which the test compound that inhibits proliferation of an
CC organism acts; (9) manufacturing an antibiotic; (10) profiling a
CC compound's activity; (11) a culture comprising strains in which the gene

CC product is overexpressed or underexpressed; (12) determining the extent
CC to which each of the strains is present in a culture or collection of
CC strains; or (13) identifying the target of a compound that inhibits the
CC proliferation of an organism. The antisense nucleic acids are useful for
CC identifying proteins or screening for homologous nucleic acids required
CC for cellular proliferation to isolate candidate molecules for rational
CC drug discovery programs, or for screening homologous nucleic acids
CC required for proliferation in cells other than S. aureus, S. typhimurium,
CC K. pneumoniae or P. aeruginosa. The present sequence is one of the target
CC prokaryotic essential genes. Note: The sequence data for this patent did
CC not form part of the printed specification, but was obtained in
CC electronic format directly from WIPO at
CC ftp.wipo.int/pub/published_pct_sequences
XX
SQ Sequence 483 BP; 134 A; 159 C; 113 G; 77 T; 0 U; 0 Other;

Alignment Scores:

Pred. No.:	3.68e-69	Length:	483
Score:	657.00	Matches:	123
Percent Similarity:	87.34%	Conservative:	15
Best Local Similarity:	77.85%	Mismatches:	20
Query Match:	75.95%	Indels:	0
DB:	7	Gaps:	0

US-09-955-315-2 (1-165) x ACA29828 (1-483)

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Qy         21 LeuGlnGluArgLeuThrAspTyrAsnAspLeuHisLeuIleLeuLysHisValHisTrp 40
              ||||||||||||||||||||||||||||||||||||||||||||||||||   ||||||
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Qy         41 AsnValThrGlyProAsnPheIleAlaValHisGluMetLeuAspProGlnValAspLeu 60
              ||||||   |||   ||||||||||||||||||||||:::||||||||||||||||||
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Qy         61 ValArgGlyTyrAlaAspGluValAlaGluArgIleSerThrLeuGlyGlyAlaProVal 80
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Qy         81 GlyThrProGluGlyHisValAlaAspArgThrProLeuGlnTyrGluArgAsnAlaGly 100
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Qy        101 AsnValGlnAlaHisLeuThrAspLeuAsnArgValTyrThrGlnValLeuThrGlyVal 120
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Qy        141 GlnAlaAlaGluLeuGluLysPheGlnTrpPheIleArgAlaHisIleValAsp 158
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Db        421 CAAGCAGCCGAACTGGAGAAATTCCAGTGGTTTATCCGCGAGCACATTGTCGAC 474
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ALIGNMENT

; Sequence 1, Application US/09738626
; Publication No. US20020197605A1
; GENERAL INFORMATION:
; APPLICANT: NAKAGAWA, SATOSHI
; APPLICANT: MIZOGUCHI, HIROSHI
; APPLICANT: ANDO, SEIKO
; APPLICANT: HAYASHI, MIKIRO
; APPLICANT: OCHIAI, KEIKO
; APPLICANT: YOKOI, HARUHIKO
; APPLICANT: TATEISHI, NAOKO
; APPLICANT: SENOH, AKIHIRO
; APPLICANT: IKEDA, MASATO
; APPLICANT: OZAKI, AKIO
; TITLE OF INVENTION: NOVEL POLYNUCLEOTIDES
; FILE REFERENCE: 249-125
; CURRENT APPLICATION NUMBER: US/09/738,626
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; PRIOR APPLICATION NUMBER: JP 00/280988
; PRIOR FILING DATE: 2000-08-03
; NUMBER OF SEQ ID NOS: 7059
; SOFTWARE: PatentIn ver. 3.0
; SEQ ID NO 1
; LENGTH: 3309400
; TYPE: DNA
; ORGANISM: Corynebacterium glutamicum
US-09-738-626-1

Query Match 100.0%; Score 1377; DB 9; Length 3309400;
Best Local Similarity 100.0%; Pred. No. 0;
Matches 1377; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

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Qy        901 TAACTGAAGACATCTACATCAGCCAGGCCGCGGAGCTGGAGAAATTCCAGTGGTTCATCC 960
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Qy       1141 ATCCACCACAGACTCCGGAATTTGCCGTGGGATGTGGTGGATCCTTGCTGTGGAACCTCA 1200
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Qy       1201 CAGGAGGTGCAAGTTGGGGGACGGAGTGTTGGGATTGCTAAAATTCGTTCGATTTTTAAG 1260
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Qy       1261 TTCTCCGCTGATCTGTAACGAGAAGTTGAACAGTCAACCTGCAGTGACCCCGCAGGAATC 1320
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Db    3200997 TTCTCCGCTGATCTGTAACGAGAAGTTGAACAGTCAACCTGCAGTGACCCCGCAGGAATC 3200938

Qy       1321 ACAGCAGCCTCAATCGCCTCAAAAGTAACGCTCCTAGAATCGCCTCTAAGGGCCTAA 1377
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    3200937 ACAGCAGCCTCAATCGCCTCAAAAGTAACGCTCCTAGAATCGCCTCTAAGGGCCTAA 3200881
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